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ABSTRACT

In this report, emphasis is placed on major research developments in the reproductive sciences, their impact on the health of individuals as well as on that of society, and on current trends that may provide new opportunities for future research in fertility and infertility. In the first section, major developments in the reproductive sciences are discussed in the areas of: (1) endocrine regulation of reproductive processes; (2) female reproductive system; (3) male reproductive system; (4) gamete biology; (5) early development of the embryo; and (6) implantation of the embryo. The second section explores the impact of recent research on health and disease. Included are discussions on: (1) male and female infertility; (2) fertility regulation; (3) relief of dysmenorrhea; (4) synchrony of estrus in animal husbandry; (5) management of breast cancers; (6) endometriosis; (7) hyperprolactinemia; and (8) disorders of sexual differentiation. The final section cites areas of future investigation in: (1) neuroendocrine control of reproduction; (2) mechanism of action of reproductive hormones; (3) reproduction peptides of gonadal origin; (4) regulation of follicular growth and ovulation; (5) regulation of fertility events in the male; (6) in vitro fertilization and embryo transfer; (7) mechanism of implantation; (8) toxins affecting reproduction; and (9) development of hybridomas. A selected bibliography of 58 references is included. (JD)

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Fertility and Infertility

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I. Introduction

Reproductive biology is the study of the transmission of life. The control of sexual differentiation and development and the means to promote reproductive health, provide adequate regulation of fertility, alleviate infertility, and treat reproductive diseases rest on a better understanding of the normal physiological mechanisms involved in the regulation of reproductive processes.

In this report, emphasis is placed on major research developments in the reproductive sciences, their impact on the health of individuals as well as on that of society, and on current trends that may provide new opportunities for future research in fertility and infertility.

II. Major Developments in the Reproductive Sciences

Endocrine Regulation of Reproductive Processes

Regulation of Gonadotropin Secretion

Mechanisms that regulate the secretion of the gonad-stimulating hormones follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary are multifactorial and may involve the central nervous system, autonomic nervous system, neuroendocrine system, and feedback action of gonadal origin. That neural stimulation exerts a profound influence over the secretion of gonadotropins is

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abundantly evident; both exteroceptive and systemic stimuli are involved.

Exteroceptive stimuli such as light, visual perception, sound, odor, and touch are known to alter gonadal function. In nearly all wild and some domesticated mammals, the breeding season is determined by the daily photoperiod. The gonads usually undergo regression during decreasing daylight and are restored under the stimulus of increased exposure to light. Some animals, such as the goat, are responsive to increased darkness. In mice, the secretion of gonadotropins is also influenced markedly by odors of the male. These olfactory stimuli can alter the timing of ovulation and terminate a pregnancy early. Among birds, egg laying is often dependent upon hearing and(or) seeing the male. Tactile stimuli govern the number of eggs laid. It is a common practice in the poultry industry to extend the photoperiod by artificial illumination as a means of increasing egg production.

There are many well-known instances of reproductive failure among mammals in captivity as a result of inadequate gonadal function. Crowding has a detrimental effect on estrous cycles and can lead to sterility in both males and females. Through this mechanism, some species are able to keep their population density at a tolerable level. Others require a high population density in order to reproduce, as did the now extinct passenger pigeon. When that population (about 4 billion) was reduced by excessive hunting for marketing purposes, the survivors were unable to reproduce either in the wild or in captivity.

In a few mammals, referred to as induced-ovulators, ovulation is dependent upon and triggered by the act of mating. Systemic stimuli arising from the cervix and other points of contact with the male activate the neuroendocrine system and bring about an ovulatory LH surge. The secretion of gonadotropin is also influenced in humans by functions of the brain. Stressful situations involving fear and severe anxiety can seriously disrupt or abolish menstrual cycles. A classic example is the amenorrhea observed in women in concentration camps during World War II. Also, in young women housed together, menstrual cycles tend to become synchronized. The nature of the stimulus is unknown, but some alteration in gonadotropic function is evident.

The neuroendocrine component of the mechanism controlling the secretion of gonadotropins involves neurosecretory neurons in the hypothalamus. A portal vascular system collects these secretions and transports them directly to the anterior hypophysis, where they stimulate gonadotropin-secreting cells to release their stored FSH and LH.

One of the major advances during the last decade was the isolation and synthesis of the polypeptide hormone, gonadotropin-releasing hormone (GnRH), produced in the brain, which stimulates the secretion of both LH and FSH. Synthesis of structural analogs of GnRH, which are 150 times more potent than the native hormone, provides a powerful tool with which to study the regulation of gonadal function. By one means or another, all of the alterations in reproductive function described above must ultimately depend upon a change in the output of gonadotropins by the pituitary. Understanding this neuroendocrine mechanism regulating gonadal function, and the means of controlling it at will, must surely stand as an achievement of incalculable benefit to humankind and a boon to the animal industry.

Feedback

Male

Secretion of the testicular hormone, testosterone, is affected by Leydig cells and regulated by LH, the secretion of which is held in check by the feedback action of testosterone. This closed-loop type of feedback control maintains the plasma level of testosterone within rather narrow physiological limits. While testosterone was formerly thought to be the major influence in regulating LH secretion, more recent studies have shown that aromatization of testosterone to estradiol in peripheral tissues accounts for most of the LH suppression. In normal men, testosterone and estradiol regulate both FSH and LH release by the pituitary, the androgen slowing the frequency of LH discharge and the estrogen damping the amplitude of the LH pulse. Both sex steroids act at the level of the hypothalamus and the pituitary; steroid receptors have been detected in each of these tissues.

The Sertoli cells in the seminiferous tubules are known to produce a second testicular hormone, inhibin, which is of peptide nature. Although inhibin has not been isolated in pure form and its physiological role remains unknown, experimental evidence suggests that it serves to restrict the secretion of pituitary FSH. The gonadotropins directly control the somatic cells of the testes (LH acting on the Leydig cells, FSH on the Sertoli cells), and they thus create the conditions necessary for normal sperm production. Androgens, estrogens, and inhibin all have the potential to directly affect the pituitary responsiveness to GnRH. Determining the respective roles of sex steroids and non-steroid testicular factors in the physiological regulation of FSH and LH secretion remains an important research goal.

In the human and in many other mammalian species, the presence of a transport protein, testosterone-estradiol-binding globulin (TEBG), modifies the interaction between the testes and the hypothalamic-pituitary axis. The transport protein is stimulated by estrogen. In states of testicular failure, when estrogen secretion is augmented, TEBG concentration increases. Both events lead to high total plasma testosterone levels and reduced free or unbound testosterone concentrations. Changes in bound and free estradiol levels also occur. Transport of sex steroids by the TEBG and albumin in circulation and their effect on unbound concentrations of testosterone and estradiol, and the clearance of these hormones into tissues, deserve further study.

Female

Regulating secretion of the two ovarian steroid hormones, estradiol and progesterone, has been the subject of intensive study for many years. This is an extremely complex mechanism; and while much has been learned, some details have not been fully clarified. The feedback actions of the ovarian steroids are of two types, negative and positive, and they have opposing end results. Negative feedback operates at all times to suppress gonadotropin, and positive feedback enhances output sharply over a very limited period of time and only in connection with the induction of the preovulatory gonadotropin surge. Unlikely as it may seem, estrogen, the most powerful suppressor of gonadotropin output known, is also the substance that triggers the preovulatory outpouring of gonadotropins. Positive feedback action always follows and supplants negative feedback action. This dual feedback system is a normal feature of the estrous or menstrual cycle of all the species that have been studied.

The feedback actions of progesterone are less well characterized than those of estrogen. Although both negative and positive feedback actions can be demonstrated experimentally, doubt remains as to the existence of a positive feedback effect under normal physiological conditions. Some evidence indicates that any negative feedback actions during the normal estrous or menstrual cycle may be of minor importance. Nonetheless, during pregnancy and lactation, the suppressive action of progesterone on LH secretion is one of the major factors responsible for inhibiting ovulation and holding cyclic function in abeyance.

Exogenously administered ovarian steroids can unquestionably inhibit the secretion of LH and ovulation. This action provides the basis for the effectiveness of oral contraceptives.

Whether the ovarian steroids act back on the hypothalamus or on the pituitary or on both has long been in question. Recent experimental evidence based on the rhesus monkey has shown that normal cyclic ovarian function is observed when the negative and the positive feedback actions of estrogen are restricted to the pituitary alone. The evidence does not rule out the possibility of some estrogenic feedback at the level of the hypothalamus, but it does tend to suggest that such is not essential.

Finally, it must be mentioned that a newly discovered peptide in follicular fluid has been found to inhibit the secretion of FSH. This peptide has been termed folliculostatin or inhibin F to distinguish it from the similar or possibly identical substance (inhibin M) found in males. Folliculostatin has proved effective in lowering the output of FSH under many different test situations, and it deserves intensive study as another possible means of controlling human fertility.

Mechanisms of Hormone Action

Peptide Hormones

Hormones in the bloodstream are distributed ubiquitously to all cells in the body, but only certain cells respond to a specific hormone. These so-called target cells, tissues, or organs have specific receptors that bind the specific hormone and decode its message. The binding sites or receptors for peptide hormones are located on the cell membrane. The resulting hormone receptor complex becomes activated and initiates a cascade of chemical processes that will eventually result in an overt expression of hormone action. Often the peptide catalyzes the enzyme adenylyl cyclase to produce cyclic adenosine monophosphate (AMP). This is the so-called "second messenger," the hormone being the "first messenger." Subsequent steps lead to the activation of protein kinases, phosphorylation of proteins, and eventually to metabolic changes necessary for cell growth and secretion. The peptide hormone itself, and perhaps the receptor as well, are internalized by endocytosis and are degraded lysosomally. Presently, there is no evidence that internalization and degradation are involved in peptide hormone action. There is much yet to be learned about the mechanism of peptide hormone action.

Steroid Hormones

In contrast to the cell membrane binding of peptide hormones, steroid hormones enter the cytoplasm where they

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are bound by specific receptor proteins. Upon binding the steroid hormone, the receptor protein becomes activated and acquires the ability to interact with one or more components of the nuclear chromatin. Within the nucleus, the receptor steroid complex promotes gene transcription of ribonucleic acid (RNA) including messenger RNA, which codes for the synthesis of new proteins. Steroid hormones thus regulate processes of growth and secretion in the accessory reproductive organs. As yet, the precise mechanism whereby the steroid receptor regulates RNA transcription remains unknown. Intensive efforts are now underway to: (1) purify the receptor in order to better understand the molecular properties that determine its interaction with the nucleus; (2) identify the specific sites of receptor interaction in the nucleus and their role in activation of gene transcription; and (3) explore possible extranuclear sites of steroid hormone action.

Female Reproductive System

Control of Follicular Maturation

The ovary has both a gametogenic function involving the production of "mature" oocytes and an endocrine function involving the production of certain "reproductive" hormones. These functions are subserved by the principal components of the ovary, including the follicular complex, the corpus luteum, and the stroma. Follicular maturation consists of developmental changes in the follicular complex that: (1) begin with the formation of the primary follicle; (2) contribute to the gametogenic and hormonal functions of the ovary; and (3) culminate in either atresia or ovulation and corpus luteum formation.

The germ cells that are destined to become oocytes make their appearance as oogonia very soon after formation of the human female embryo. From an initial count of approximately 1,700, the oogonia increase to approximately 600,000 during the 6th to 8th week of pregnancy, and to almost 7 million by the 20th week. Oogonia reside in relatively undifferentiated structures in the ovary known as primordial follicles.

Follicular maturation, without ovulation, begins before birth when some of the oogonia enter into the process of meiosis (or reductional division), lose forever their capacity to produce additional oogonia from mitosis, and are transformed in the process to oocytes. The relatively undifferentiated primordial follicle is transformed into a primary one with the first appearance of a layer of granulosa cells around the oocyte. These developments mark the

beginning of follicular maturation, which before menarche leads to degeneration of the follicular complex or atresia, and after menarche, to atresia or ovulation. Through the process of atresia, the population of approximately 7 million eggs is reduced to 1 to 2 million by birth, and to approximately 400,000 by menarche, when the first ovulation occurs.

In the adult, the process of follicular maturation can be divided into preantral, antral, and preovulatory stages. The preantral follicular complex is derived from the primordial follicle and oogonium and consists of an oocyte and a single layer of granulosa cells. Although FSH appears to be involved, the mechanism by which follicular maturation is initiated by conversion of the primordial follicle to a primary follicular complex is unknown. It does appear, however, that, once initiated, follicular maturation is sequential and continuous until atresia or ovulation occurs.

The formation of a fluid-filled cavity, or antrum, in the follicle is the principal indication of the progression of the follicular complex from the preantral to the antral stage. This progression is also characterized by a marked increase in the number of granulosa cells, an increase in the size of the oocyte, completion of a structure around it called a zona pellucida, further development of the theca in the stroma immediately surrounding the follicle, and an overall increase in the size of the follicular complex. On the basis of recent advances in knowledge of events that transpire within the follicle (follicular environment), it is possible to distinguish between those antral follicles having high and those having low probabilities for continued maturation. The following conditions characterize antral follicles with a high probability for continued maturation and maintenance of healthy oocytes: (1) full complement of granulosa cells; (2) high estrogen content or high estrogen-to-androgen ratio; and (3) presence of detectable amounts of FSH in the antral fluid. When these conditions do not occur in the follicular environment, some degree of atresia is usually evident, with accompanying degeneration and possible necrosis of the resident oocyte. It appears that FSH from the pituitary gland plays a key role in the support of follicular maturation, particularly during its antral stage. There is considerable evidence that FSH stimulates the production of estrogen by the follicular complex. This estrogen, perhaps in combination with FSH, helps to maintain the full complement of granulosa cells in the follicle through stimulation of their multiplication. Increasing levels of estrogen from antral follicles stimulated by FSH and LH serve to terminate the menstrual period and initiate the follicular phase of the menstrual cycle.

The preovulatory stage of follicular maturation is the last one before the process is terminated either by ovula-

tion and corpus luteum formation or by atresia and "death" of the follicle. During this stage, there is a substantial increase in the quantity of follicular fluid in the antrum and in the overall size of the follicular complex. The egg assumes an eccentric location in the follicle, is surrounded by a layer of cells called the corona radiata, and may be either free-floating in the follicle or attached by a thin stalk, the cumulus oophorus. Presumably, as a result of its release from some inhibitor present in the follicle, the oocyte resumes meiosis and sustains it until ovulation occurs. This period of follicular maturation is characterized by the appearance of the "dominant" follicle, which is the only preovulatory or graafian follicle in the cohort that is destined to ovulate. Current evidence suggests that atresia is the fate of all of the remaining follicles in this cohort.

The process of follicular maturation is brought to a dramatic conclusion by the rupture of the follicle and release of the egg in the process of ovulation. LH (a second gonadotropic hormone secreted by the pituitary gland) is believed to play a principal role in the control of the preovulatory stage of follicular maturation and in ovulation itself. When the follicle is suitably prepared, ovulation occurs following a "surge" in the release of LH from the pituitary gland. LH facilitates the preparation of the cohort of follicles destined to enter the next cycle of follicular maturation, and may also facilitate the completion of meiosis in the oocyte by somehow releasing it from the control of a meiotic inhibitor. As its presence in the follicular environment diminishes, FSH plays a much less dominant role in the events of this stage than it did in the previous one.

Over and above the endocrine events that are mediated centrally, a number of local ovarian factors influence ovulation. The ovarian stroma is endowed with smooth muscle elements that surround the follicles and may play a role in ovum expulsion. An in vitro model has been developed that allows serial observation of follicular development and ovulation, isolated from systemic influences, and evaluation of the direct effect of various agents on the process. The rupture of mature ovarian follicles can be induced in the rabbit by prostaglandin F-2 α . Indeed, prostaglandin inhibitors such as indomethacin inhibit ovum expulsion both in vivo and in vitro. Prolactin inhibits ovulation in vitro despite ovarian stimulation with human chorionic gonadotropin (HCG). This finding suggests that prolactin can influence ovulation directly at the ovarian level. Since antihistamines and chelating agents also inhibit ovulation, ionic calcium and histamine are implicated in the ovulatory process.

Knowledge of how follicular maturation is controlled, and of how 1 percent of follicles may be selected for ovulation while the remaining ones proceed to atresia and death, is not only essential for an understanding of female reproduction, but also promises to suggest new measures for the alleviation of infertility as well as for the regulation of fertility. Some forms of infertility appear to be related to failure of follicular maturation. Suppression of dominant follicle formation could lead to the development of a method of fertility regulation based on 100 percent follicular atresia rather than the 99 percent that occurs normally in the ovary.

Regulation of the Corpus Luteum

After ovulation, the ruptured follicle is converted into a ball of cells known as a corpus luteum. The corpus luteum is the major source of sex steroid hormones secreted by the ovary during the postovulatory phase of the menstrual cycle. In the absence of pregnancy, the functional life of the corpus luteum is 14 ± 2 days, after which it regresses or degenerates into a nonfunctional mass of tissue called a corpus albicans. Progesterone, which is one of the hormones secreted by the corpus luteum, plays a key role in the regulation of the postovulatory phase of the menstrual cycle, and if a fertilized egg is implanted in the uterus, in the establishment and maintenance of pregnancy.

The regulation of the corpus luteum is dependent upon the pituitary gland. While FSH undoubtedly plays a role, LH is the principal agent responsible for the conversion of the postovulatory follicle into a corpus luteum. In the typical human female reproductive cycle, the corpus luteum appears to have a finite life, as evidenced by signs of regression and deterioration at approximately 10 to 12 days after ovulation. Unless pregnancy intervenes, this regression and deterioration continue until the 12th to 14th day, when insufficient progesterone is produced to maintain the luteal phase of the cycle and the menstrual period begins. When pregnancy intervenes, the corpus luteum is "rescued" by a hormone secreted by the developing placenta, HCG. HCG, chemically and functionally related to LH, has the capacity to stimulate the corpus luteum to secrete progesterone far beyond the time it normally would under the influence of LH in the typical cycle.

The mechanism of regulation of the finite life of the corpus luteum is a major unanswered question in the reproductive sciences. While prostaglandin(s) has been implicated as the agent(s) responsible for the termination of

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luteal responsiveness to LH, there is no conclusive evidence for this action in the human female cycle.

Male Reproductive System

Sexual Differentiation

Sexual differentiation of the male reproductive system is a complex series of events, beginning with induction of the gonad and proceeding through maturation of the testes and formation of the genitalia. The genetic sex of the embryo is determined at fertilization when the X-bearing oocyte fuses with an X- or Y-bearing sperm to form the zygote. Differentiation of the gonad and the genital apparatus, however, requires a further complex series of interdependent genetic, endocrine, and biochemical events. Beginning about the 7th week of the embryonic period, the indifferent gonad evolves into a testis by an interaction of the primordial germ cells with the epithelial cells of the urogenital ridge. By the 8th week, the testis consists of cords of germ cells and Sertoli cell precursors that will form seminiferous tubules and interstitial cells, and will later form the Leydig cells capable of androgen secretion.

Recent evidence suggests that a gene normally on the Y chromosome is responsible for differentiation of the indifferent fetal gonad, which otherwise becomes an ovary, into a testis. The inducer is the "HY antigen," a cell surface antigen present only on male cells: a testis cell suspension from newborn rats can reorganize spontaneously into testicular tissue, but fails to do so when these cells are exposed to HY antibodies, and an ovary is formed instead. Conversely, suspensions of newborn rat ovarian cells, after addition of HY antigen, reorganize into testicular structures. Under special circumstances, HY antigen may be expressed in the absence of the Y chromosome (probably due to translocation of the gene to another chromosome). This absence leads to XO or XX phenotypic males. In vitro bioassay of the HY antigen has revealed a remarkable evolutionary conservation of the HY antigen among male cells of all mammalian species tested so far, including humans, while female cells of the same species possess no cross-reacting plasma membrane component. There is a gonadal receptor for HY antigen. Fetal ovarian cells exposed to soluble HY take up the molecule and bind HY antibody; specific uptake does not occur in the extragonadal tissues. It may be inferred that HY antigen is disseminated during embryogenesis and bound by specific receptors in cells of the primordial gonad, and that reaction of HY and its receptor signals a program of testicular differentiation regardless of karyotype. The several anomalies of primary sexual differentia-

tion manifest in such conditions as the XX male, the XX true hermaphrodite, and thus the XY female can reasonably be viewed as specific errors of synthesis, dissemination, and binding of HY antigen.

The embryonic testis is hormonally active. It not only secretes a factor (müllerian-inhibiting substance--MIS) that triggers the regression of the müllerian ducts, which otherwise would form fallopian tubes and uterus, but also secretes testosterone, which stimulates the wolffian ducts to differentiate into vas, epididymis, seminal vesicles, and ejaculatory ducts. Testosterone also virilizes the urogenital sinus and genital tubercle. This virilization leads to the formation of prostate, penis, and closure of the labial scrotal folds to form the scrotum. While testosterone per se is required for testis maturation, wolffian and external genital maturation requires conversion of testosterone to dihydrotestosterone for androgen action at those tissue sites.

The early phase of testis development is not influenced by the fetal pituitary. While GnRH has been detected in the fetal hypothalamus as early as the 8th week, gonadotropin secretion by the fetal pituitary does not begin until the 10th week, and it reaches a maximum at midpregnancy. Thus, even in fetuses in which formation of the hypothalamus and pituitary is abnormal, differentiation of internal and external genitalia progresses normally. During early embryonic development, testosterone secretion is regulated by chorionic gonadotropin from the placenta. The precise age when fetal pituitary gonadotropin secretion comes under control of the gonadal sex steroids is not known.

The mechanism of testicular induction of müllerian regression has also been of considerable interest. The müllerian-inhibiting substance appears to be produced by the fetal Sertoli cell soon after differentiation of the seminiferous tubule. The substance is recognized throughout the fetal life and the early neonatal period. While the structure of the molecule has not been elucidated, it appears to be a glycoprotein, possibly composed of subunits. Characterization of the events surrounding müllerian regression has been slowed by the cumbersome bioassay required to demonstrate its presence. With the use of hybridoma techniques, however, a monoclonal antibody has been raised against MIS, making radioimmunoassay feasible.

Regulation of Spermatogenesis

The spermatogenic process is a complex event under the control of gonadotropic hormones FSH and LH from the pitui-

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tary. In this process the primitive germ cells multiply and establish renewable stem cells from which mature sperm evolve. Initiation, completion, and maintenance of spermatogenesis are entirely under endocrine control. In human subjects with an isolated deficiency of the two gonadotropins FSH and LH, the testes remain infantile at the level of the 7-month fetus. While recent evidence indicates that testosterone is important for initiation of spermatogenesis, determining the precise role of FSH and other factors in this process will require further study.

For the human, while testicular function begins in early pregnancy, germinal maturation normally begins in late pregnancy. Thereafter, the testes enlarge in proportion to the multiplication of germ cell content and growth of the seminiferous tubules. GnRH is detectable in the fetal hypothalamus, and gonadotropin release is evident in the late fetus and early neonatal child. Following these first few months of postnatal life, however, the infant enters a latent period that ends with the onset of puberty. In most species testicular maturation is complete at a very young age, but in the human male, puberty is a relatively late event, beginning at approximately age 11 to 14 years.

The spermatogenic tissue lies within the seminiferous tubules of the testis. The seminiferous epithelium has a number of unique characteristics among epithelia. It is composed of a nondividing population of supporting Sertoli cells and a proliferating population of germ cells. The Sertoli cells extend the full thickness of the epithelium while the germ cells occupy interstices between the supporting cells and slowly move toward the lumen of the tubules, where they are eventually exfoliated. Specialized junctions between Sertoli cells divide the seminiferous epithelium into a basal compartment containing stem cells and an adluminal compartment containing the differentiating germ cells. The stem cells in the basal compartment are exposed to the same fluid environment as the interstitial tissue; the differentiating germ cells, however, are isolated from it by the intraepithelial barrier. This blood-testis barrier forms early in testicular development, after which permeability of the Sertoli cells is limited. The occurrence of two populations of germ cells, one fixed and the other moving upward, located in two distinct epithelial compartments separated by a permeability barrier (the blood-testis barrier) is a feature unique to the seminiferous epithelium.

In contrast to the development of ova, spermatogenesis is a continuously active process, except in seasonal-breeding mammals. The process of stem cell renewal and the spermatogenic cycle have been the subject of considerable research. The time required for progression of a primitive

spermatogonium to a mature sperm is a precisely timed event. In the human this requires 74 days. Among the important gaps in knowledge is a lack of understanding of the factors that determine when a stem cell will divide, and whether the cellular progeny will renew the stem cell pool or take the path of further differentiation.

Meiosis is a special type of cell division that occurs in germ cells and leads to formulation of gametes containing one-half the number of chromosomes (haploid) present in somatic cells (diploid). It is during this stage that the important interchange of genetic material takes place between pairs of homologous chromosomes. This process of recombination allows a single gamete to contain genetic information derived from both parents. Knowledge about the meiotic process and the mechanisms that regulate it stems from studies of lower species. Little, if any, information is available in the primate and human.

Leydig cells are the site of sex steroid synthesis and secretion. These cells are interspersed between the tubules containing the germ cells in such a way that release of androgens and, to a lesser extent, estrogens, bathes the tubules in a high concentration. Because capillaries are in close proximity to the Leydig cells, the sex steroids are released into the general circulation for distant effects. Studies in the rodent and human have shown that testosterone synthesis is due to the action of LH upon the Leydig cell. LH binds to plasma membrane receptors and activates cyclic AMP, leading to protein and steroid biosynthesis. LH accelerates the conversion of cholesterol to pregnenolone in the biosynthetic pathway. In some species prolactin may play a synergistic role. While no one has yet shown that FSH binds to the Leydig cell, it is clear from the studies in animal and man that FSH augments Leydig cell secretion of testosterone in response to LH. The mechanism of this interaction is unclear.

FSH binds to specific plasma membrane receptors of the Sertoli cell and activates cyclic AMP and protein synthesis. These events are most evident in the immature testis and are least apparent after gonadal maturation is complete. A number of actions have been demonstrated for FSH at the level of the Sertoli cell. Protein synthesis is increased, and the cytoskeleton and shape of the Sertoli cell are modified in response to FSH. Glucose, vitamin A, calcium, and other cofactors are important in this process. Under FSH stimulation, Sertoli cells secrete an androgen-binding protein along with numerous other proteins that may serve as "regulating factors" in various stages of germ development.

In addition to FSH, Sertoli cell functions are regulated by androgens. A specific testosterone receptor has been

identified in Sertoli cells, and it is likely that the effects of androgen on the germ cell are mediated by Sertoli cells. Further studies will be needed to define the precise hormonal requirements for initiation and maintenance of the spermatogenic process.

Gamete Biology

Advances in basic knowledge of the hormonal regulation of the gonads have clarified the role played by the brain and the pituitary gland in the development of the gametes--that is, spermatozoa in the testis and oocytes in the ovary. Once released from the gonads, the male and female gametes enter a phase of limited lifespan. Although there remain many unresolved problems related to this phase of development, important advances have been made recently in the understanding of their transport, maturation, and fusion.

Sperm Maturation

Spermatozoa are released from the seminiferous tubules and transported from the testis through the rete testis into a system of excurrent ducts. This system is comprised of several ductuli efferentes that empty into a single, highly convoluted ductus epididymidis; this, in turn, is continuous with the straight ductus or vas deferens. In most mammalian species, including the human male, sperm transport through the epididymis takes approximately 10 to 15 days. During this time spermatozoa develop the capacity for motility, and change from functionally immature cells unable to fertilize an egg to cells with full fertilizing potential.

This process of physiological maturation is accompanied by a series of morphological, metabolic, and biochemical changes. The most obvious morphological changes are the migration of the cytoplasmic droplet and the reorganization of the acrosome to a more compact form. Their functional significance is unknown. Some of the known metabolic and biochemical changes accompanying sperm maturation include an increase in glycolysis, respiration, stabilization of the structural elements of the sperm by oxidation of the sulfhydryl groups into disulfide bonds and intracellular content of cyclic AMP and carnitine, and a decrease in synthetic activities and intracellular content of phospholipid and protein.

Changes have also been observed in the physiochemical characteristics of the plasma membrane. Modifications of

the plasma membrane are of special interest because gamete recognition and fusion are events involving the membrane. Ultrastructural investigations have revealed specific differences in the configuration of the plasma membrane between immature and mature epididymal spermatozoa. The macromolecular composition and properties of the plasma membrane also change during epididymal transit. These changes include adhesiveness, accumulation of a net negative surface charge, changes in the density and distribution of lectin-binding sites, and changes in the antigenic properties of the sperm plasma membrane. The character and properties of the sperm plasma membrane are not uniform over the entire sperm surface, but show distinct regional characteristics. The significance of such regional specialization is unknown; it may determine, however, the functional specificity of restricted regions of the sperm surface. The use of biochemical probes to label selectively the surface components of the spermatozoa has provided direct evidence for changes in the composition of sperm plasma membrane during sperm maturation. For example, spermatozoa from the distal epididymis, but not from the proximal epididymis, possess an externally oriented plasma membrane glycoprotein of 37,000 daltons. Its appearance on the sperm surface coincides with the development of fertilizing ability. Sperm maturation in the excurrent duct is therefore a complex process in which many aspects of the structure, biochemistry, and physiology of the spermatozoa change toward a final mature state. Some of these changes are intrinsic to the sperm, but others are dependent upon the epididymal environment.

The development of the fertilizing ability of the spermatozoa in the proximal epididymis and their survival in the distal epididymis depend upon the environment created by the synthetic activities of the epididymis, which are androgen-dependent. The development of the fertilizing ability of the spermatozoa, for example, could be blocked in vitro by preventing interaction of androgen with its receptor or by blocking RNA and synthesis of protein by the epididymal epithelium.

The development of motility, which is another aspect of the physiological maturation of the sperm, is also dependent upon contributions by the excurrent duct. Spermatozoa released from the proximal epididymis swim in circles; those taken from the distal epididymis exhibit normal forward motility. Forward motion can be induced in immature spermatozoa by the addition of seminal plasma and of theophylline, which elevates the intracellular concentration of cyclic AMP. A component of the seminal plasma required for these motility effects has been identified as a glycoprotein, and partially purified. It is found in highest concentration in fluid from the distal epididymis, and is also found in the

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seminal plasma of a variety of animals. Cross-reactivity between species has been demonstrated by the ability of bovine sperm to be activated by the seminal plasma of several mammalian species, and by the ability of bovine seminal plasma to induce forward motility in immature sperm in a number of mammals, including man. It follows that exposure to the epididymal environment is necessary for the production of highly motile cells having the capacity to fertilize ova.

The protein composition of the fluids varies from the proximal to the distal epididymis. The chemical components are concentrated, reabsorbed, and secreted by the epididymal epithelium. With the exception of the protein responsible for induction of forward motility, however, these components are of unknown function. The interaction of spermatozoa with the fluids of the reproductive tract can modify the metabolism and the surface of the spermatozoa. For example, carnitine, which is not synthesized by the epididymis but is derived from the blood, reduces metabolic activity in spermatozoa. It is present in high concentrations in fluids of the distal epididymis, and may be related to the long-term storage known to occur in this part of the duct. There is also evidence that the sperm surface can be modified by interaction with luminal fluids during epididymal transit; components associated with sperm membranes can be removed, added, or modified.

A gap in understanding the maturational process of the spermatozoa is the functional significance of many of the observed biochemical and structural changes undergone by spermatozoa as they pass through the epididymis. A thorough understanding of the mechanisms by which substances from the male genital tract modify sperm characteristics may lead to recognition and possible alleviation of some form of male infertility or subfertility. It would also offer more sensitive and specific methods to regulate fertility.

Sperm Capacitation

Even after maturation in the epididymis, spermatozoa still have not attained their full capacity for fertilization. After they enter the female reproductive tract, they undergo further physiological changes that make them able to penetrate the zona and fuse with the egg. This final preparation for fertilization is called capacitation. The period of time required for capacitation is necessary for the successful initiation of the sperm acrosome reaction, and it varies considerably, depending on the animal. Events that occur during capacitation include increases in sperm

respiration, glycolysis, and motility, loss of the protective investment of glycoprotein molecules from the sperm surface, changes in membrane fluidity, and subtle ultrastructural changes in components of the membrane.

The biochemical mechanisms responsible for the capacitation process in mammals are not fully understood. Studies of invertebrates have provided valuable insights into the fundamental mechanisms of fertilization. It has been known for more than 60 years, for example, that a soluble component associated with sea urchin eggs stimulates sperm respiration rates. Recent studies have shown that this respiration-stimulating factor is a peptide of low molecular weight; it stimulates the oxidation of fatty acids to CO_2 , and the motility of spermatozoa. This peptide also causes increases of both sperm cyclic AMP and cyclic guanosine 3',5' monophosphate (GMP) concentrations, and continues to produce all its effects in the absence of extracellular calcium.

In mammals, fertilization occurs in the ampulla of the oviduct. Luminal fluids from the oviduct, as well as follicular fluid, have been tested for effects on sperm metabolism. Although a positive effect has been consistently reported, characterization of the molecules responsible for the sperm metabolic effects has not yet been achieved because of the wide range of substances present in oviductal fluid.

Capacitation is a reversible phenomenon. Capacitated spermatozoa can be decapacitated upon exposure to seminal plasma and recapacitated following their deposition in the female genital tract. The chemical nature of the decapacitation factor is unclear. It has been reported to be a glycoprotein of high molecular weight; another study implicates the lipid fraction of membranous vesicles present in the seminal plasma. Extraction of cholesterol and phospholipid from these vesicles has decreased their decapacitation activity. In contrast, proteolytic digestion designed to remove surface proteins from the vesicles has had no effect. After exposure to vesicles, the ratio of cholesterol to phospholipid is increased in plasma membranes. Such an increase would stabilize the membrane and prevent its fusion.

Gamete Interaction

The study of invertebrate gametes has been invaluable for understanding mammalian fertilization. Detailed analyses of fertilization in sea urchins have revealed the nature

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of the molecular interactions between the two gametes and have provided the foundation for current concepts of fertilization.

The binding of sea urchin spermatozoa to the egg investments is one of the initial steps in fertilization. The fertilizing spermatozoon is almost always one of the first to attach to the surface of the egg. All spermatozoa rotate around the point of attachment for about 20 seconds and then suddenly stop moving and rest perpendicular to the egg's surface. Almost immediately, the acrosome reaction occurs and the first response of the egg is seen as the cortical reaction begins and the fertilization membrane around the fertilizing sperm is elevated.

The acrosome reaction involves a progressive organized membrane fusion that occurs between the plasma membrane and the underlying outer acrosomal membrane leading to vesiculation and the release of acrosomal contents. One of the released components is a trypsin-like enzyme required for fertilization. The component responsible for induction of the acrosome reaction in the sea urchin is a large macromolecule that contains principally fucose and sulfate. This factor increases sperm cyclic AMP in a calcium-dependent manner. The primary effect of this factor appears to be on transport of calcium and subsequent modification of various enzymes, including adenylate cyclase, which distinguishes it from the low molecular weight peptide that stimulates sperm respiration and motility. How calcium exerts its effects on the metabolism of sperm cyclic nucleotide and the specific mechanism by which cyclic AMP and(or) calcium are involved in the acrosome reaction are speculative at this time. The polymerization of actin and the formation of microfilaments during the acrosomal reaction may present a potential site of cyclic AMP action. In sea urchins, therefore, two distinct components associated with the egg induce capacitation (increased respiration and motility) and the acrosomal reaction, and might represent the physiological initiators of these events in vitro.

The acrosomal reaction uncovers an egg-binding protein of approximately 30,500 daltons, called bindin. Bindin interacts with a specific carbohydrate receptor on the egg membrane. This sperm receptor is a glycoprotein of high molecular weight with a high species-specific affinity for bindin. Sperm and egg interact by a process of membrane fusion whereby a patch of sperm membrane remains on the egg and persists throughout early development. Fusion triggers a release of calcium from the egg, beginning at the point of sperm penetration and spreading over the entire surface of the egg. This calcium wave triggers exocytosis of cortical granules and the formation of the fertilization membrane, which prevent additional penetration by spermatozoa.

In mammals, fertilization occurs within the oviduct. The successful development of techniques for in vitro fertilization has permitted a direct observation of this process. Binding of a spermatozoon to the surface of the zona pellucida occurs through the plasma membrane overlying the acrosome, and the spermatozoon remains attached for at least 15 minutes before penetrating the zona pellucida. The acrosomal reaction probably occurs simultaneously with this initial binding. Exocytosis releases hydrolytic enzymes, including acrosin, which are believed to be essential for penetration of the investments of the eggs. Proacrosin is found in the acrosome of mammalian spermatozoa in its inactive zymogenic form, and is converted to acrosin during capacitation. A uterine glycosaminoglycan stimulates the conversion of highly purified proacrosin to acrosin. The mechanism for this activation is unknown, but may represent one of the physiological changes undergone by the sperm during capacitation.

Questions concerning which components of the reproductive tract trigger the acrosomal reaction have not been resolved. Based on in vitro studies, albumin (a protein of high molecular weight from the serum) and an immunological complement present in follicular fluid have been proposed as the agents responsible for the induction of the acrosomal reaction. Actin is present in vertebrate spermatozoa, but whether or not it undergoes polymerization during a normal acrosomal reaction has not been reported. Formation of microfilaments, however, has been reported after a chemically induced acrosomal reaction. Calcium is essential for the acrosomal reaction of mammalian spermatozoa in vitro, and cyclic nucleotides also appear to be involved. Activation of protease and phospholipase influx of calcium, efflux of H^+ , other ionic fluxes, and activation of adenosinetriphosphate (ATPase) have all been suggested as primary events in the acrosomal reaction. There is no compelling evidence at present for a single biochemical mechanism for the regulation of the mammalian acrosomal reaction.

It had been reported from observations on fixed and sectioned eggs that sperm penetrate the zona pellucida at an oblique angle and fuse with the vitelline membrane through the postacrosomal membrane. Direct observation, however, shows that mammalian spermatozoa penetrate the zona pellucida, pass through the perivitelline space, and fuse with the vitelline membrane at an angle perpendicular to the surface. Penetration of the zona is accomplished in approximately 20 minutes and passage through the vitelline space in less than a second. As the spermatozoon passes through the zona pellucida, the flagellum is highly motile. As soon as the tip of the sperm head contacts the vitellus, however, the flagellum becomes immotile and the sperm head gradually sinks into the vitellus. The cortical granules are dis-

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charged and the ensuing reaction inhibits penetration of the zona pellucida and the vitellus by additional sperm. A receptor exists in the zona pellucida for capacitated sperm. It is destroyed by proteases and is probably a protein, but it remains to be characterized. It is not known whether the equivalent of bindin exists in mammalian sperm.

Sea urchin fertilization and mammalian fertilization have strong similarities and identical mechanisms. For a fuller understanding of the molecular events in mammalian fertilization, it will be necessary not only to study mammalian fertilization but also to continue the study of nonmammalian species, particularly marine invertebrates.

Early Development of the Embryo

A series of crucial events occurs after fertilization, and if they are successful, they result in the formation of a new organism. This is a vulnerable period, when as many as one-half of all human pregnancies terminate spontaneously and early. The developmental events that take place within the maternal organism have two separate programs. The first focuses on the transport of the embryo through the fallopian tube into the hormonally prepared uterus, and the second on the molecular events within the zygote, beginning with establishment of conditions conducive to interaction of maternal and paternal DNA and subsequent cleavage.

Zygote Transport

Although fertilization takes place within the fallopian tube, the embryo, which has no means of self-propulsion, must be moved into the uterine cavity. Tubal transport depends on maternal hormonal status, which influences tubal structure and function. The fallopian tube has several possible mechanisms for zygote transport, including tubal muscles, microvilli, cilia, and tubal secretions. After ovulation, tubal smooth muscle contractions and cilia pick up the oocyte from the surface of the ovary. The oocyte is transported rapidly by tubal cilia to the ampullary-isthmic junction. After being held at the ampullary-isthmic junction for 3 days, during which time fertilization occurs, the zygote is rapidly transported into the uterus. At the present time, a detailed understanding of the control and integration of transport mechanisms is lacking.

Although the flow of tubal secretions may influence tubal transport of the embryo, its major function appears to be nutritive.

The extent to which disordered tubal transport causes human infertility is unknown. Premature entry of the embryo into the uterus in nonhuman primates prevents implantation. Failure to move the embryo into the uterus by the time of implantation results in an ectopic pregnancy. Ectopic pregnancies, with rare exceptions, terminate in the demise of the embryo and threaten the life of the mother. Ectopic pregnancies are clinically associated with previous tubal infections and endometriosis, disorders that disrupt tubal anatomy or physiology. The full role of the multiple etiologies resulting in ectopic pregnancy, however, has yet to be characterized.

Biology of the Blastocyst

The fertilized egg, or zygote, contains all the genetic information necessary for the development of an individual. A series of molecular events occurs after fertilization that allows interactions between the maternal and paternal DNA, setting in motion the programs of cell division, motion, and differentiation responsible for the formation of a multicellular organism from a single cell. The understanding of early differentiation of the embryo is a direct result of advances in cell biology and embryo culture techniques.

After fertilization, decondensation of paternal DNA, and formation of male and female pronuclei, the maternal and paternal chromosomes are brought together to form a diploid cell. As expression of embryonic messenger RNA does not occur until after the eight-cell stage, early molecular events depend on maternal macromolecules contained in the oocyte. Organization of ooplasm and differential distribution to cells in early cleavage stages, however, do not appear to influence differentiation.

The first differentiation of embryonic cells is commitment to formation of inner cell mass, or trophectoderm. Commitment occurs after the eight-cell embryonic stage and appears to depend on cell location in the blastomere. Embryonic cells up to the eight-cell stage are loosely associated, none being completely on the inside or outside of the blastomere. After the eight-cell stage, there are cells that are on the inside or outside of the blastomere. The cells on the outside form tight junctions with each other during compaction of the blastomere. After compaction, the blastocyst forms with two distinct lines of cells,

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trophectoderm outside, the inner cell mass inside. Although further development of the trophectoderm depends on the presence of the inner cell mass, removal of the trophectoderm does not appear to block placentation and embryonic development.

Differentiation of cells in the inner cell mass to form fetal and extraembryonic cells also appears to depend on cellular location. Prior to commitment of inner cell mass cells to ectodermal or endodermal differentiation, the inner cell mass also undergoes a period of compaction. The presumption is that external cells will become ectodermal while inner cells will be progenitors of endodermal structures.

During this period of time, molecular events also change drastically in the developing embryo. Proteins synthesized in the early embryo are similar to those found in the mature oocyte. By the eight-cell stage, however, embryonic proteins are expressed. By the time the blastocyst forms, specific proteins of trophectoderm and inner cell mass are found. Therefore, although early events in the embryo depend on maternal proteins and messenger RNAs, the embryo quickly expresses its own genes and determines cellular differentiation.

Implantation of the Embryo

Following ovulation and during the time that the ovum resides in the oviduct, becomes fertilized, and initiates cleavage divisions, the uterine lining undergoes extensive morphological and biochemical changes in preparation for receiving the fertilized ovum. Such changes include the activation of protein and carbohydrate secretory apparatus in epithelial and glandular cells, amplification of the endometrial vascular bed, and early changes ("predecidual") in certain stromal cell populations. Mitotic proliferation of epithelial components precedes the appearance of differentiation of these cells and is generally regarded as being due to estrogen stimulation. Mitosis of stromal cells and the differentiation of epithelial components apparently require progesterone or both progesterone and estrogen. In the human uterus, concentrations of cytoplasmic receptors for estrogen begin to rise shortly after menstruation. Following estrogen interaction with cytoplasmic and nuclear receptors, estrogen-dependent proteins are synthesized, one of which is the receptor for progesterone. Thus, by the time of ovulation and subsequent production of progesterone by the corpus luteum, endometrial cells have an increased number of progesterone receptors.

Secretory material produced by the uterus under progesterone stimulation includes one or more specific uterine proteins or glycoproteins that may be of significance to the maintenance of the blastocyst. One such protein, uteroglobin, has received considerable attention in recent years; however, its precise role in early pregnancy has yet to be elucidated. Other products of the hormone-primed endometrium include increasing concentrations of several proteolytic enzymes. One proposed role for these proteases is the lysis of blastocyst coverings (perhaps including the zona pellucida), from which the blastocyst must be freed prior to adhesion with the uterine epithelium. Drug-induced inhibition of protease activity reportedly delays or interrupts the implantation process. It seems likely that uterine proteases and other lysosomal hydrolases play an important role in implantation.

The blastocyst is metabolically active during the immediate preimplantation period. In addition to the synthetic processes necessary for its own growth and differentiation, blastocysts of several species are known to elaborate products that may be regarded as hormonal signals to the uterus. The "maternal recognition of pregnancy" has been described as the changes in maternal physiology, brought about in response to an embryo, that are necessary for the survival of the embryo. Evidence for estrogen production by early embryos of certain species has been obtained in the past few years. Other embryos, such as human, are known to produce gonadotropins. The chorionic gonadotropin (HCG) secreted by trophoblastic cells is believed to be responsible for "rescuing" the corpus luteum, the demise of which would trigger loss of the uterine lining and, of course, terminate the pregnancy.

Blastocyst adhesion to the epithelium of a receptive uterus involves membrane-membrane interactions. In many species studied in this regard, trophoblastic cells appear to form junctional attachments to uterine epithelial cells followed by fusion of the two cell types, advancement of the trophoblast towards subjacent vasculature by penetration around epithelial cells, or advancement of trophoblast accompanied (or preceded) by displacement and loss of uterine epithelial cells. Hormone- or blastocyst-induced changes in the luminal plasma membranes of the epithelial cells have been investigated with respect to changes in surface charge and in the disposition of lectin-binding sites. In at least one species, uterine epithelial cells have a net negative charge on their surface membranes at the time of ovulation. The anionic character of the cell coat is reportedly lost by the time implantation occurs; the loss perhaps facilitates cellular adhesion to the blastocyst. There is at present, however, no consensus as to the mechanism of cellular adhesion at implantation.

Endometrial tissue reacts to the implanting blastocyst by exhibiting a physiological response similar to acute inflammatory reactions. For many years investigators have disputed a potential role for one vasoactive mediator of inflammation, histamine, in the implantation process. Recent studies have revived this issue and provided convincing evidence that another group of mediators, the prostaglandins, are involved in the increases in blood flow and plasma leakage from endometrial vessels at the site of implantation. The uterine response culminates in the formation of a new type of tissue, the decidual tissue. The decidua may form somewhat of a buffer zone between the developing placenta and the deeper layers (myometrium) of the uterus.

A major thesis in the biology of implantation as derived from animal studies appears to be the ultimate requirement for synchronous development of the blastocyst and the endometrium. The uterus is surprisingly refractory to ovo-implantation, except for a brief period reached after proper hormonal priming and differentiation of endometrial cells. Likewise, temporal incompatibility for implantation could result from advanced or retarded development of the blastocyst, which results in improper embryo-to-uterus hormonal "signals" or modulation of membrane surface properties. If this requirement for synchronous development exists in humans, a number of events conceivably could go awry and result in infertility. The same events, if controlled, could assist in the regulation of fertility.

III. Impact of Recent Research on Health and Disease

Advances in both basic and clinical reproductive biology have greatly increased the scope of treatable disorders of the reproductive tract. Broader understanding of the hormonal regulatory events involved in the menstrual cycle has led to drugs capable of inducing ovulation in noncycling women. Similarly in the male, fundamental studies of reproductive function have led to successful treatment of men with deficiency of the pituitary hormones and allowed for reversal of infertility. This information also provides a data base upon which to develop male contraceptives. The definition of the molecular mechanisms of steroid hormone action has led to a methodology that enables the physician to select proper treatment for women with breast cancer. The risks to both mother and infant of childbearing at the early and late extremes of reproductive life are known to increase. A notable impact of both basic

and clinical research in reproductive sciences has been new knowledge on methods to regulate fertility.

Major advances in health care that derive from support of fundamental research are highlighted below.

Female Infertility: Induction of Ovulation

An estimated 15 percent of couples are infertile or subfertile. One cause of infertility is failure to ovulate or infrequent ovulation. Knowledge of hormonal events during the menstrual cycle has led to an understanding of the control of ovulation. Women with an intact, responsive pituitary can be treated successfully with clomiphene, which stimulates pituitary release of gonadotropins and results in ovulation. Women with an unresponsive pituitary can be treated with gonadotropins for direct ovarian stimulation and ovulation.

Male Infertility: Stimulation of Spermatogenesis in Men With Deficiency in Gonadotropic Hormones

From studies in animals and man, it is now known that spermatogenesis requires high levels of both testosterone and FSH. While FSH and testosterone act directly on Sertoli cells, which nourish the developing germ cells, FSH also enhances Leydig cell secretion of testosterone (mechanism unknown). The availability of human gonadotropins has made it possible to treat hypogonadotropic men. Not only do such patients virilize, but in addition, they ultimately produce numbers of sperm adequate to impregnate their partners. The recent availability of GnRH and highly potent GnRH analogs offers an alternative approach to stimulating spermatogenesis through their ability to stimulate endogenous pituitary gonadotropin release. Only preliminary studies of the use of these compounds in humans have been reported. The results suggest that, to be effective, subcutaneous delivery systems capable of releasing GnRH in a regular pulsatile fashion will be needed, because authentic GnRH requires multiple daily injections and the potent analogs "down-regulate" the pituitary-gonadal axis.

Fertility Regulation: Oral Contraception in Females

One of the major advances resulting from research in steroid hormone action and steroid biochemistry was the

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development of oral contraceptives. Successful, safe, and reliable fertility regulation has been one of the major medical advances of the 20th century. Current studies are clarifying risk factors in the use of oral contraceptives and may lead to more stringent criteria for use as well as to the design of safer formulations.

Relief of Dysmenorrhea

Dysmenorrhea is a major reproductive disorder that accounts for untold hours of pain, lost work, and disrupted schedules. Research on prostaglandins has led to an understanding of the mechanism of dysmenorrhea. The development of prostaglandin synthetase inhibitors, which are often successful in treating dysmenorrhea, is a major advance in reproductive medicine.

Synchrony of Estrus in Animal Husbandry

Domestic animal production for milk and meat is dependent upon reproduction. Recent advances in the understanding of hormonal factors involved in ovulation and embryo transfer may lead to significant improvement in availability of meat and dairy products from domestic livestock.

Management of Breast Cancers

Breast cancer is the most common malignancy in women in North America. One of thirteen women will develop the disease sometime during her life. Clinically, it has been known for many years that breast cancers vary in response to hormonal therapy. Elucidation of the mechanism of steroid hormone action has identified cytoplasmic receptors as the transducer of the hormonal message. Subsequent studies have demonstrated the relationship between the presence of estrogen receptors and hormonal responsiveness of tumor cells. Only 12 percent of patients whose tumors lacked significant estrogen receptor levels responded to hormone treatment, whereas 50 percent of those with estrogen receptors responded to hormone treatment. The ability to predict the response of a given breast tumor to endocrine therapy was further refined after the demonstration that estrogen initiated the synthesis of progesterone receptors; 77 percent of

patients whose tumors were positive for both estrogen and progesterone receptors benefited from endocrine therapy. This knowledge has altered the treatment for women with estrogen-responsive breast tumors and has replaced surgical endocrine ablation with estrogen antagonist therapy. Further research may result in development of cytostatic agents with receptor affinity and may lower the generalized toxicity of these agents.

Endometriosis

Endometriosis is a disease characterized by the presence and proliferation of endometrial tissue outside the uterus. It is found in 14 to 21 percent of all gynecologic operations, and 30 to 40 percent of patients with endometriosis have concomitant infertility. Although the etiology of this disease is still under debate, recognition that the lesion is ectopic endometrial tissue (which like endometrium is responsive to estrogen and progesterone) has led to the development of a rational hormonal treatment. Studies demonstrating progesterone inhibition of estrogen receptor production and endometrial cell atrophy have led to long-term treatment with progesterone or estrogen-progesterone combinations. This therapeutic modality results in variable degrees of success in control of pain and alleviation of infertility. Also, development of Danazol, which, among its other effects, inhibits gonadotropin secretion, has contributed to the treatment of this reproductive disorder.

Hyperprolactinemia

Prolactin is unique among the pituitary hormones in that its secretion is controlled predominantly by an inhibiting factor rather than a releasing factor. In mammals, prolactin is essential for milk production. Hypersecretion of prolactin may result from several physiological or pathological conditions, including pregnancy, pituitary tumor, or drugs.

Hyperprolactinemia produces galactorrhea and interferes with gonadal function in women. Nonpregnant patients with hyperprolactinemia and no evidence of a pituitary tumor may be successfully treated with drugs such as bromocriptine. Although such drugs may prove useful in treating certain patients who have pituitary tumors, the long-term management of these patients is still not resolved.

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Disorders of Sexual Differentiation

The major benefit of research in this area has been the acquisition of a firm body of genetic, endocrine, and embryologic information upon which to construct a rational basis for the diagnosis and therapy of disorders of sexual differentiation. The presence of a testis, but the genital apparatus of a female, is known as "male pseudohermaphroditism." Such errors in sexual differentiation are now known to be due to either defective action of androgen in the target tissues, defective testosterone biosynthesis, or failure to reduce testosterone to dihydrotestosterone, which is the active metabolite in the genital tissues.

Because tests and reagents are now available to measure abnormalities in steroid hormone formation and abnormalities of steroid entry into target tissues, it is now possible to establish the etiology of ambiguous genitalia and assign a proper sex gender. Surgical correction of the abnormality and hormonal replacement therapy can permit normal sexual function later in life, although fertility may not be restored.

IV. Areas for Future Investigation

It is clear that many fundamental questions in reproductive biology remain unresolved. The research areas identified below appear to give promise for the development of new means to regulate fertility, alleviate infertility, and treat diseases related to reproduction. It is recognized, however, that advances in science do not follow a predictable course. More often than not, breakthroughs are unexpected. Hypothalamic-releasing factors, for example, were thought to exert their action only through the pituitary gland. It was not foreseen, prior to the availability and study of synthetic GnRH analogs, that these compounds could act directly on the gonads and that some of the antifertility effects observed after administration of GnRH analogs resulted from this direct action. This unexpected observation has broadened the understanding of gonadal regulation and may lead to improved methods of fertility regulation.

Progress in science comes not only from the recognition of a need for a better understanding of a biological process, but also with the availability of new technologies that permit further investigations of that process. In this framework important research areas, in terms of perceived need or opportunity, are suggested.

Neuroendocrine Control of Reproduction

GnRH Analogs

Numerous GnRH analogs have been produced and many more will be forthcoming. Some, the agonists, are 50 to 150 times more active than the native decapeptide. Others inhibit the native peptide and are termed antagonists. Both are of immense interest with respect to the regulation of human fertility. Agonists given in low doses, by the pulsatile mode of administration, stimulate gonadotropin output. At higher doses, with daily administration, they act paradoxically to induce gonadal regression, inhibit ovulation, or shorten the luteal phase. Some of these suppressive outcomes are due, at least in part, to the direct inhibitory action of GnRH on the gonads themselves.

Therefore, more fundamental work on GnRH analogs needs to be carried out. The use of these compounds that modulate pituitary release of gonadotropins and act directly on the gonads may in shadow the development of potent contraceptive drugs, as well as improved therapy for certain types of infertility.

Opiates

The recent increasing interest in the opioid peptides is fully justified in view of two findings: (1) the opioid peptides exist as component parts of the amino acid chain of a precursor molecule, β -lipotropin, and are derived therefrom; and (2) receptors exist for these hitherto unknown biological entities, indicating that they are part of normal neural or neuroendocrine physiology.

All of the opioid peptides are found in the brain or spinal cord except one, β -endorphin, which is primarily located in the hypothalamus and anterior pituitary, and is secreted, along with adrenocorticotrophic hormone (ACTH), in response to stress. The exogenous administration of some of these opioids has produced dramatic behavioral changes and incomparable relief of pain. The endocrine consequences of stress are of potential importance to reproductive endocrinology. The capacity of stress to perturb and even suspend menstrual function has long been known, and is implicit in the terms "boarding school amenorrhea" and "psychogenic amenorrhea." Pregnancy and the menopause are, of course, the commonest causes of amenorrhea. So-called "hypothalamic amenorrhea," generally traceable to stress, undernutrition, or both, ranks next. Opiate drugs have the capacity to

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influence reproductive function in both sexes, a property that their endogenous counterparts may well share. Evidence regarding this hypothesis is currently being sought by means of the administration of Naloxone, an antagonist of opiates and endogenous opioids. This agent has been found to increase LH secretion in women with certain types of hypothalamic amenorrhea, in normal prepubertal rats of both sexes at specific stages of puberty, and in adult male rats.

Therefore, the endogenous opioids may play a physiologic role in normal sexual maturation in addition to exerting pathological effects in the induction of stress-induced reproductive disorders. The elucidation of their role in reproductive endocrinology awaits further explorations in this newly opened frontier in biomedical science.

Dopamine

Dopamine is a neurotransmitter that may modulate the secretion of some of the hypothalamic hormones. There is good evidence that dopamine can inhibit the secretion of prolactin, but whether it acts as a neurotransmitter or as a hypothalamic hormone is unknown. Some maintain, but others disagree, that dopamine is the well-documented prolactin-inhibiting factor (PIF).

Further study of the catecholamines as neurotransmitters in the regulation of lactation, galactorrhea, amenorrhea, and other neuroendocrine disorders is of urgent importance.

Mechanism of Action of Reproductive Hormones

The mechanism of action of reproductive hormones is at once the most important and elusive goal of scientists engaged in basic research on reproduction. Scientists and physicians would be in a much better position to correct undesirable hormone-mediated actions and to induce desired ones if they understood the mechanisms by which such actions in the body are mediated under normal circumstances. There is considerable difficulty, however, in obtaining in an appropriate form sufficient amounts of those cellular components and substances required for studies of the mechanism of hormone action.

Overwhelming evidence indicates that estrogen controls the production of a protein called ovalbumin by "turning on"

ovalbumin genes in the body. The mechanism by which the ovalbumin gene is regulated by estrogen cannot be determined with conventional methods; it would be necessary to obtain reproductive tracts from thousands of animals to obtain enough cells with the required genes. Even if enough cells could be obtained, however, the ovalbumin genes cannot be separated from the thousands of other genes with which they are associated in such cells.

Previously impossible goals in research on the mechanism of hormone action have become feasible and attainable because of the truly remarkable progress made during the last 6 or 7 years in the new field of recombinant DNA technology. With the advent of the technology, scientists are able to analyze, duplicate, or recombine the genetic material DNA, which serves as a basis of all living organisms, to attain a variety of ends and products. The recombinant DNA "tools" enable scientists to obtain copies of virtually any given gene, to increase the numbers of such genes to any amount desired through amplification in bacterial hosts, which serve as "living factories," and to obtain unlimited amounts of proteins whose synthesis is directed by such genes. One specific implication of this new technology involves research on progesterone, sometimes called the hormone of pregnancy. Pregnancy does not occur in the absence of progesterone. Since progesterone is believed to exert its actions in the body primarily through gene regulation, the new recombinant DNA technology promises to reveal a means to prevent undesired pregnancies through interference with the normal gene-regulating activities of progesterone, as well as a therapy to facilitate a pregnancy in a woman whose infertility is caused by insufficient progesterone or its inappropriate action in the body.

The implications of recombinant DNA technology are that further study could lead to alleviation or cure of infertility and reproductive diseases and the development of new, safe, inexpensive, and efficacious measures for fertility regulation.

Reproductive Peptides of Gonadal Origin

Studies of putative gonadal peptides comprise one of the most rapidly growing and potentially significant areas of reproductive biology. Recently discovered gonadal peptides have been found to inhibit FSH secretion (inhibin/folliculostatin), GnRH stimulation of gonadotropin secretion (gonadostatin), oocyte maturation (oocyte maturation inhibi-

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tor), granulosa cell luteinization (luteinization inhibitor), LH binding to target cells (LH binding inhibitor), and FSH binding to granulosa and testicular cells (FSH binding inhibitor). Since none of these gonadal peptides has been structurally determined and synthesized, it is quite likely that different names have been given to the same peptide and that there are fewer of these peptides than this list would suggest.

The gonadal peptides that have received the most attention are inhibin/folliculostatin and the oocyte maturation inhibitor. Both are promising candidates for a second generation of contraceptive agents based on highly selective inhibition of a specific reproductive event or process rather than the reprogramming of virtually all of the interrelationships of female endocrinology. For example, a contraceptive agent that acts by inhibiting FSH secretion selectively might not have the undesirable side effects that are currently attributed to the pill. Similarly, an agent that prevents oocyte maturation may exert only a purely local effect on the oocyte.

Studies in this area are needed to accelerate the design of satisfactory assays for the putative peptides and the isolation, purification, structural determination, and synthesis of the compounds.

Regulation of Follicular Growth and Ovulation

Ovulation was recognized very early as one of the reproductive processes most amenable to intervention for fertility regulation. Despite a wealth of information, many questions remain unanswered. For example, the dynamics of follicular growth and atresia are not fully understood. The mechanism that determines which oocytes and follicles will be stimulated to grow and mature within each menstrual cycle is not known, nor is the mechanism of follicular atresia. The latter appears to be a normal process that occurs in all species studied. The factors responsible for atresia from the fetal period until the onset of puberty and then continuing within each menstrual cycle are poorly defined.

In the female, the egg is arrested in its development. An inhibitor produced by the follicle cells surrounding the oocyte is responsible for keeping the oocyte from maturing and extruding the first polar body. Characterization and elucidation of the mode of action of this intraovarian factor controlling maturation of the follicles are essential to understanding female reproduction.

Although detailed information on the endocrine events that precede ovulation is available, the events that occur locally within the ovary have been relatively neglected. A variety of substances have been shown to have a local effect on ovulation. The observation that prostaglandin inhibitors, such as indomethacin, actually prevent the expulsion of an egg suggests the possibility of a pharmacological approach to ovulation suppression which could work without any influence on systemic endocrine events and would therefore cause minimal disturbance of the menstrual cycle. Investigation of these local biochemical mediators of ovulation may lead to further insights regarding the causes of reproductive failure.

Determination of the mechanism for follicular growth, oocyte maturation, follicular atresia, and ovulation offers significant opportunities to develop new methods for alleviating some form of infertility or controlling fertility.

Regulation of Fertility Events in the Male

New knowledge of the regulation of testicular function and sperm transport now offers a wide variety of potential sites of attack for a male contraceptive. Heretofore, attempts have been made to suppress the production of sperm using injectable, long-acting male hormones, analogous to the approach used in the suppression of ovulation in the female with the pill.

An important problem encountered with long-acting testosterone preparations is that these drugs do not maintain their desired action long enough and separate from other effects. In too many instances, sperm production breaks through after a few weeks or months; further, the hormone has to be injected intramuscularly. The use of oral progestins, synthetic androgens, or GnRH antagonists to inhibit spermatogenesis is an attractive concept, but when these drugs are administered alone they reduce sexual interest significantly and thus require additional administration of a potent, long-acting injectable androgen. At present, the development of new contraceptive methods for men seems distant and will require an innovative approach.

Research over the past several years has identified the Sertoli cell as a mediator of hormone action on the germinal epithelium. Sertoli cells are controlled largely by FSH and androgenic hormones. In response to hormonal stimulation, they secrete numerous proteins. It now appears likely that certain of these proteins function as "promoting factors" to regulate key steps in the formation of spermatozoa; Sertoli

cell function varies in different segments of the seminiferous tubule depending on the stage of spermatogenesis with which they are associated. For example, secretion of androgen-binding protein is most active in association with the formation of mature spermatids. This fluctuation in Sertoli cell function suggests that germ cells may also exercise local control over the function of Sertoli cells. Technology has now advanced to a point where it is possible, with the use of in vitro systems, to isolate and determine the specific function of yet-unknown Sertoli cell-promoting factors.

It now seems feasible to consider other mechanisms for male fertility regulation, such as altering epididymal sperm maturation or sperm motility. Recent research findings increasingly support the idea that the changes taking place in the spermatozoon during its transit through the male and female genital tracts, which are relevant to its fusion with the egg, occur at the level of the plasma membrane. Although some of these changes may be independent of the environment, others are evoked by interaction with environmental factors in both male and female tracts.

An understanding of Sertoli cell-germ cell interaction in the testis and sperm maturational changes in the epididymis will mark a major breakthrough in designing methods of controlling fertility and treating disorders of male infertility.

In Vitro Fertilization and Embryo Transfer

Research on in vitro fertilization and embryo transfer provides scientists with a unique tool to study an organism at its very origin. By studying the sperm and egg in controlled isolation, scientists can examine gamete structure and biology, observe the crucial, yet poorly understood, mechanisms of fertilization, and understand genetic exchange and sex determination. Such research can help clarify the changes that must occur in sperm to render it capable of fertilization, penetration of the egg, and the means by which the egg protects itself against fertilization by more than one sperm. As the transition from fertilized gamete to embryo begins, researchers can study early cellular division, a process that is basic to the development of an individual. Such studies may someday provide a basis for the diagnosis and prevention of congenital or developmental defects.

Fertilization and embryo transfer have been achieved in vitro for most of the animals commonly used in biomedical research. Until recently, in vitro fertilization had not

been successful in subhuman primates. Scientists have now succeeded in fertilizing the eggs of rhesus monkeys in vitro and transferring them to three rhesus monkeys that presently carry viable fetuses. This new development is quite significant because, unlike some subhuman primates, the female rhesus monkey has a menstrual cycle and reproductive organs similar in many respects to those of women.

The procedure of embryo transfer is highly developed and widely used in domestic animals. Embryos that develop after artificial insemination of cattle of preferred genetic constitution, for example, are routinely transferred to other suitably prepared cattle, which serve as foster mothers for their development, birth, and nursing. When relieved of these responsibilities through embryo transfer, the cattle with preferred genetic characteristics have markedly increased productivity of embryos, because they can be inseminated more frequently than if they were required to carry their young to term.

The procedures of in vitro fertilization and embryo transfer may be of possible use in helping women with certain tubal defects bear their own children. In a classic case, Dr. Patrick Steptoe and Dr. Robert Edwards of Great Britain reported that one of their patients gave birth to an apparently normal baby that developed from an egg fertilized in vitro. According to Drs. Steptoe and Edwards, the procedures were required because the mother's fallopian tubes were blocked and therefore normal or in vivo fertilization could not occur.

More research in the area of in vitro fertilization and embryo transfer in animals is needed to determine whether the procedure is repeatable and whether it leads to abnormal offspring.

Mechanism of Implantation

The series of events whereby genetically different cell types (trophoblast and uterine epithelium) interact and result in implantation remains relatively unexplored. Investigations of embryonic and maternal cell interaction using laboratory animal models must be continued, and additional studies on primates should be encouraged. Prospective investigations should also reevaluate the thesis of embryo-uterine synchrony required for successful implantation, particularly with respect to primate models.

A better understanding of the requirements for embryonic and endometrial synchrony would be extremely important in the area of embryo transfer and might provide new information on infertility.

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Toxins Affecting Reproduction

During the past decade, the medical community has become increasingly aware of the adverse effect of many drugs and environmental toxins upon the reproductive processes of both men and women. The reproductive processes are particularly susceptible to injury. Chemical agents may affect not only gonadal and genital maturation of the developing fetus, but also the germ cell content of the testis and ovary, and in extreme cases they may produce a state of functional castration.

Among patients surviving cancer as well as other chronic diseases, many of the agents used for treatment leave the survivor sterile as a result of depletion of the germ cells within the gonad. In women, premature menopause may occur; in men, gynecomastia and impotence are occasionally seen. These effects depend on which drugs are used and on the total dose administered. The more common offenders include external radiation, alkylating agents, methylhydrazines, and most recently, doxorubicin. Occupational exposure to kepones, dibromochloropropane (DBCP), polycyclic biphenyls (PCBs), and possibly lead, boron, and carbon disulfide may similarly lead to serious deterioration of gonadal function. Among men, there is suggestive evidence that increased alcohol ingestion and smoking of marihuana can lead to some sexual dysfunction. Deterioration of gonadal function can be insidious in the male, and sterility may occur following drug or toxin exposure, while libido and potency are preserved.

Further broad emphasis on reproductive toxicology is necessary, particularly with respect to the effects of environmental toxins and drugs used to treat chronic illness.

Development of Hybridomas

Development of the radioimmunoassay has been crucial for the advancement of knowledge in reproductive endocrinology. These sensitive immunoassays have allowed hormonal and gonadotropin events of the menstrual cycle to be characterized, and have led to the present understanding of the mechanisms of control along the hypothalamic-pituitary-gonadal axis. The antisera used for these assays, however, are relatively crude, and they contain many different antibodies directed at different regions of the stimulating antigen that elicited their formation. Sera formed in one animal can change with time over the life of the animal, and may be difficult or impossible to reproduce in another animal.

Advancements in molecular biology, immunology, and cancer biology have resulted in the hybridoma technique, which is a potent new biotechnology. With this technique an animal is injected with an antigen to obtain antibody-producing spleen cells to be fused with a cancerous cell (myeloma). The resulting hybrid cell produces the single type of antibody molecule of its spleen cell parent but continues to multiply like its myeloma cell parent. The offspring of this fusion, a hybridoma, is a transformed cell that has an apparently unlimited lifespan and produces a single antibody.

Hybridomas producing single monoclonal antibodies in culture represent clean, renewable antibody production systems of enormous potential for reproductive biology.

Conclusion

The accomplishments in reproductive research and advances gained in understanding the reproductive process during the past decade have been enormous; oral contraception, induction of ovulation, and management of breast cancer are only examples of the benefit derived from such studies. The major advances that have been made in the understanding of reproductive processes and in reproductive medicine have been the result of basic and clinical research. The future of fertility regulation, the management of infertility, and the management of reproductive disorders will depend on basic and clinical research and research training in the reproductive sciences.

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